Changes in endogenous bioactive compounds of Korean native chicken meat at different ages and during cooking

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ABSTRACT This study aimed to examine the effect of bird age on the contents of endogenous bioactive compounds, including carnosine, anserine, creatine, betaine, and carnitine, in meat from a certified meat-type commercial Korean native chicken strain (KNC; *Woorimatdag*). Additionally, the effects of the meat type (breast or leg meat) and the state of the meat (raw or cooked) were examined. Cocks of KNC were raised under similar standard commercial conditions at a commercial chicken farm. At various ages (10, 11, 12, 13, and 14 wk), breast and leg meats from a total of 10 birds from each age group were obtained. Raw and cooked meat samples were then prepared separately and analyzed for bioactive compounds. The age of the KNC had a significant effect only on the betaine content. The breast meat of KNC had higher amounts of carnosine and anserine but had lower amounts of betaine and carnitine than the leg meat ($P < 0.05$). The KNC meat lost significant amounts of all bioactive compounds during cooking ($P < 0.05$). Leg meat had high retention percentages of carnosine and anserine after cooking, whereas breast meat showed almost complete retention of betaine and carnitine. The results of this study provide useful and rare information regarding the presence, amounts, and determinants of endogenous bioactive compounds in KNC meat, which can be useful for selection and breeding programs, and also for popularizing indigenous chicken meat.

Key words: age, Korean native chicken, cooked meat, dipeptide, betaine

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INTRODUCTION

Recent research has highlighted the vital role played by some of the endogenous bioactive compounds of meat in human health. Lee and Ho (2002) defined a bioactive component of a diet as any food or part of a food that provides medical or health benefits, including prevention and treatment of a disease. Carnosine, anserine, creatine, taurine, ubiquinone, betaine, and carnitine are some of the common endogenous bioactive compounds available in meat (Purchas et al., 2004; Mora et al., 2008; Rigault et al., 2008; Alirezaei et al., 2012; Peiretti et al., 2012).

Carnosine is a naturally occurring histidyl dipeptide (*N*-β-alanyl-L-histidine) with several biological functions. It has been credited with a potent buffering role (Intarapichet and Maikhunthod, 2005), antioxidant properties (O'Neill et al., 1999), and antiaging properties (Purchas et al., 2004). In addition, Peiretti et al. (2011) reported that carnosine possesses defense mechanisms against glycation and oxidation, which are related to diabetes, kidney diseases, and some forms of cancers. Carnosine is widely distributed in vertebrate animal tissues, including skeletal muscle (Mora et al., 2008). Anserine (β-alanyl-1-methyl-L-histidine), another histidyl dipeptide, is present in higher levels in the skeletal muscle of most vertebrates, including turkey, chicken, and rabbit, but is absent in the same muscles of humans (Peiretti et al., 2011). Similar to carnosine, this bioactive compound has buffering capacity and antioxidant properties (Peiretti et al., 2012). The nitrogenous organic acid creatine [*N*-(aminoiminomethyl)-*N*-methyl-glycine] can be synthesized in the kidneys, pancreas, and liver from L-arginine, glycine, and L-methionine (Schmid, 2009; Mora et al., 2010). Creatine plays a vital role, together with creatine phosphate, in the energy metabolism of skeletal muscle (Wyss and Kaddurah-Daouk, 2000). Hence, athletes use creatine to improve performance capability (Volek and Rawson, 2004). Furthermore, Adhihetty and Beal (2008) reported neuroprotective effects of creatine. Some evidence exists that creatine has sensory properties contribut-

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ing to the full flavor of meat extracts (Cambero et al., 2000; Mora et al., 2010).

Carnitine (γ-trimethylamino-β-hydroxy butyric acid), a lysine-derived molecule, is important in animals due to its important function in fatty acid metabolism. It transports long-chain fatty acids from the cytosol into the mitochondrial matrix for β-oxidation (Arslan et al., 2003). After distributing to organs and tissues, such as muscle and heart, they are used for energy production. Carnitine is supplied to the human body through different food sources, in addition to biosynthesis in the kidneys, liver, and brain (Rigault et al., 2008). It has also been suggested as an alternative therapy in patients with certain diseases, such as Alzheimer's disease and chronic fatigue syndrome (Li et al., 2007). Betaine (*N*-*N*-*N*-trimethylglycine) is a small zwitterionic compound that has osmoregulatory properties; it can be biosynthesized by bacteria, plants, invertebrates, and mammals (de Zwart et al., 2003). In addition, betaine can improve growth performance and fat distribution. It functions as a methyl donor within tissue, and is thereby useful in the synthesis of methionine, phosphatidylcholine, and creatine, which are the key components in protein and energy metabolism in cells (Alirezaei et al., 2012).

Over the years, indigenous chicken meat in different countries has attracted domestic consumers to a greater extent compared with commercial broiler meat (Wattanachant et al., 2004). Similarly, Korean native chicken (**KNC**) has become popular among Korean consumers because of the unique flavor and texture of the meat (Jayasena et al., 2013). Many researchers have conducted studies to compare the quality characteristics of KNC and broiler meats during the past few years; however, information regarding the presence or amount of the above mentioned bioactive compounds in KNC meat is scarce. Recently, Jung et al. (2013) compared the contents of bioactive compounds in meat from different lines of KNC. However, the effect of bird age on the contents of these bioactive compounds in meat and the availability of these compounds after cooking have not been well established for KNC. Therefore, the main objective of the current study was to examine how the contents of carnosine, anserine, creatine, betaine, and carnitine in KNC meat are affected by the age of bird, cooking, and meat type (breast vs. leg meat).

MATERIALS AND METHODS

For all experimental protocols, recommendations described in *The Guide for the Care and Use of Laboratory Animals*, published by the Institutional Animal Care and Use Committee of the National Institute of Animal Science (2012) in Korea, were followed. In addition, the chicken care facilities and the procedures were conducted to meet or exceed the standards established by the Committee for Accreditation of Laboratory Animal Care at the National Institute of Animal Science in Korea.

Birds

Fifty cocks from a certified meat-type commercial KNC strain (*Woorimatdag*) were used to determine the histidyl dipeptides, creatine, betaine, and carnitine contents in this experiment. During the experimental period, KNC were raised under similar standard commercial conditions at a commercial chicken farm (Gimcheon, Korea) with good husbandry conditions (i.e., litter, ventilation, stocking density). A total of 80 one-day-old chicks (*Woorimatdag*) were first obtained from a local hatchery and allotted to 5 floor pens (16 chicks/pen) within a single house. Chicks were fed commercial starter (3,100 kcal of ME/kg, 23% CP during the first wk), grower (3,200 kcal of ME/kg, 20% CP from the second to third weeks), and finisher (3,200 kcal of ME/kg, 18% CP from the fourth wk to respective age) diets ad libitum and had free access to water during the whole experiment period. The litter was checked and maintained daily and additional materials were added if the birds were slightly dirty. The birds had no access to the outdoor environment.

Processing

At each of the 5 ages tested in this study (10, 11, 12, 13, and 14 wk), 2 KNC were randomly selected from each of the 5 pens (a total of 10 KNC from each age) and subjected to a 10-h feed-withdrawal period. Subsequently, KNC were exsanguinated by a conventional neck cut and were bled for 2 min. The carcasses were then defeathered and eviscerated manually. The sex of the birds was further confirmed during evisceration to avoid any sex effect on the parameters tested in this study. The carcasses were chilled at 4°C for 24 h followed by vacuum-packing and storage in a freezer at −20°C until further analysis.

Raw and Cooked Sample Preparation

Frozen carcasses of each treatment were thawed in a refrigerator (4°C) for 24 h. Each thawed carcass was split into halves. Both the breast and leg meat were carefully dissected from the left half of each carcass and used to prepare the raw meat samples. After trimming the visible skin, fat, and connective tissues, the left breast and leg meat from each carcass were minced separately using a minichopper (CH180, Kenwood, Shenzhen, China) and used for the analysis.

The remaining 10 halves from each treatment were used for the preparation of cooked meat samples. They were boiled separately in water (1:1.5 wt/vol) for 40 min until a core temperature of >72°C was reached, which represented the domestic boiling conditions for chicken meat. The temperature of the meat was measured by a digital thermometer (YF-160A Type-K, YFE, Taiwan). The carcasses were then removed from the boiling water, vacuum packed, and cooled under running water. Finally, the cooked breast and leg

meat from each half of the carcasses was dissected and deboned separately and used for analysis after manually chopping into small pieces.

Carnosine, Anserine, and Creatine Contents

The contents of carnosine, anserine, and creatine were measured according to the method described by Mora et al. (2007). Raw and cooked meat samples (2.5 g) of each chicken were homogenized separately with 7.5 mL of 0.01 *N* HCl at 13,500 rpm for 1 min [T25b, Ika Works (Asia)., Sdn, Bhd, Malaysia]. The homogenate was centrifuged at 17,030 × *g* for 15 min at 4°C (HM-150IV, Hanil Co., Ltd., Inchun, Korea), and the supernatant (250 μL) was mixed with 750 μL of acetonitrile. After storage at 4°C for 20 min and centrifugation at 10,000 × *g* for 10 min at 4°C (HM-150IV, Hanil), the supernatant was injected into an HPLC column with a Waters 1525 pump and a Waters 717 plus autosampler (Millipore Co-Operative, Milford, MA). An Atlantis HILIC silica column (4.6 × 150 mm, 3 μm, Millipore) was used. A diode array detector (Millipore) was used at 214 nm to determine the creatine, carnosine, and anserine contents. Mobile phase A was 0.65 m*M* ammonium acetate in water and acetonitrile (25:75 vol/vol, pH 5.5), and mobile phase B was 4.55 m*M* ammonium acetate in water and acetonitrile (70:30 vol/vol, pH 5.5). Mobile phase B was supplied at 1.2 mL/min for 16 min with a linear gradient (0 to 100%). The contents of the compounds were calculated using a standard curve for each compound. Standards (carnosine, anserine, and creatine) were obtained from Sigma Co. (St. Louis, MO).

Betaine and Carnitine Contents

The betaine and carnitine contents in the raw and cooked meat samples were determined by the method of Li et al. (2007) with some modifications. Raw and cooked meat samples (3 g) of each chicken were homogenized separately with 10 mL of precipitating reagent (acetonitrile/methanol solution, 9:1 vol/vol) at 13,500 rpm for 30 s [Ika Works (Asia)]. The homogenate was then centrifuged at 2,090 × *g* for 5 min at 4°C (HM-150IV, Hanil), and the supernatant was filtered into a 20-mL volumetric flask through a funnel plugged with glass wool. After adding 10 mL of precipitating reagent to the remaining filtrate, the above procedure was repeated and the supernatant was collected in the same volumetric flask. The volumetric flask was then filled with the precipitating reagent, and 2 mL of this sample was mixed with 0.810 g of Na_2HPO_4 and 0.090 g of Ag_2O (9:1 wt/wt) in a 15-mL tube by vigorous shaking and vortex-mixing. Each mixture was air-dried in a shaker without caps for 30 min and then centrifuged (Hanil) at 2,090 × *g* for 5 min at 4°C. An aliquot of 0.5 mL supernatant was mixed with 0.5 mL of derivatizing reagent (1.39 g of bromoacetophenone and 0.066 g of 18-crown-6 in 100 mL of acetonitrile) in a 15-mL tube and vortex-mixed. The mixture was heated at 80°C for 60 min in a water bath and cooled for 5 min under running water. The sample solution was filtered through 0.2-μm membrane filters and injected into an HPLC column with a Waters 1525 pump and a Waters 717 plus autosampler (Millipore). An Atlantis HILIC silica column (4.6 × 150 mm, 3 μm, Millipore) was used. A diode array detector (Waters 2487, Millipore) was used at 254 nm to determine the betaine and carnitine contents. Mobile phase A was 25 m*M* ammonium acetate in formic acid (pH 3.0), and mobile phase B was acetonitrile. The mobile phase was supplied at 1.4 mL/min for 20 min with an isocratic elution (90% A; 10% B). The betaine and carnitine contents were calculated by using a standard curve of each compound. Standards (betaine and L-carnitine hydrochloride) were obtained from Sigma Co.

Statistical Analysis

Multifactorial ANOVA using the general linear model was conducted for the analysis of all data to confirm the effect of the age of the KNC (10–14 wk), the meat type (breast and leg), and the state of the meat (raw and cooked meat). After grouping the data by each state of meat with each meat type, the data were analyzed by one-way ANOVA using the general linear model to confirm the age effect in each state of meat with each meat type and the meat-type effect in each state of meat with age. Mean separation was conducted using Tukey's multiple range test ($P < 0.05$). The mean values and SEM were reported. The SAS software (version 9.3, SAS Institute Inc., Cary, NC) was used for all statistical analyses.

RESULTS AND DISCUSSION

Carnosine Content

Table 1 shows the carnosine content of breast and leg meat of KNC before and after cooking. Chan and Decker (1994) reported that the carnosine content in poultry skeletal muscle increased as the age of the birds increased. However, no significant age effect on the carnosine content of the breast and leg meat of KNC, either in raw or cooked meat, was found in the present study. The carnosine contents of raw meat from KNC varied from 160 to 201 mg/100 g of breast meat and 55 to 88 mg/100 g of leg meat (Table 1). Similar carnosine contents in raw meat from 5 lines of male KNC (Jung et al., 2013) and black-bone silky fowl (Tian et al., 2007) have been reported. Higher carnosine contents compared with the present study were also found both in raw breast (621 to 818 mg/100 g) and thigh (271 to 363 mg/100 g) meat from male Thai indigenous and hybrid native chickens (Intarapichet and Maikhunthod, 2005), which might be attributed to the breed effect (Abe and Okuma, 1995).

Table 1. Carnosine content (mg/100 g) of the raw and cooked meat of Korean native chicken at different ages

	Raw meat			Cooked meat			Analyzed value		
Item (mg/100 g)	Breast	Leg	SEM[1]	Breast	Leg	SEM[1]	Meat type	State of meat	Age
Age (wk)									
10	189.60[a]	86.80[b]	16.83	161.28[a]	50.09[b]	22.10			
11	160.00[a]	87.60[b]	18.56	101.83	66.06	24.54			
12	182.40[a]	55.20[b]	10.48	163.04[a]	46.25[b]	19.74			
13	200.80[a]	62.40[b]	11.71	181.07[a]	66.33[b]	26.63			
14	179.60[a]	68.00[b]	13.97	103.95	55.50	30.99			
SEM[2]	16.22	12.86		36.45	7.03				
P-value							<0.0001	0.0039	0.3173
F-value							112.82	9.03	1.21

[a,b]Mean values in the same row with different superscripts within the same state of meat differ significantly ($P < 0.05$).

[1]n = 20.

[2]n = 50.

For all age groups and in both states of meat, the breast meat of KNC showed significantly higher carnosine contents (average of 162.24 mg/100 g) compared with the leg meat (average of 64.42 mg/100 g), except in the cooked meat from the 11- and 14-wk-old birds (Table 1). The breast meat had approximately 2 to 3 times higher carnosine contents than did the leg meat (Table 1). This result was in agreement with previous findings by several researchers, who explained that white muscles contained higher carnosine contents than dark muscles (Davey, 1960; Intarapichet and Maikhunthod, 2005; Maikhunthod and Intarapichet, 2005; Tian et al., 2007; Jung et al., 2013). This can be explained by the different composition of muscle fibers in breast and leg meat. Breast meat is composed of more than 90% of white fibers (type IIB muscle fibers), whereas leg meat mainly contains red fibers, which are mainly type I muscle fibers (Lengerken et al., 2002; Intarapichet and Maikhunthod, 2005). Type IIB muscle fibers, as opposed to type I, depend primarily on anaerobic metabolism for adenosine triphosphate generation, which ultimately increases the chance of lactic acid accumulation (Maikhunthod and Intarapichet, 2005). Hence, breast muscles require large amounts of histidyl dipeptides, such as carnosine (Dunnett and Harris, 1995), which possess good buffering potential in the physiological range of pH due to accumulation of lactic acid (Davey, 1960). In addition, Sewell et al. (1992) found a strong positive correlation between carnosine content and type IIB fiber in the equine middle gluteal muscle.

Furthermore, the difference in carnosine contents between raw and cooked meat of KNC was more clear-cut (Table 1), as raw meat had significantly higher carnosine contents (average of 127.24 mg/100 g) than cooked meat (average of 99.43 mg/100 g). Purchas et al. (2004) found a similar cooking effect (boiling in water) on the carnosine contents of longissimus and semimembranosus muscles of lambs. Furthermore, Peiretti et al. (2012) showed the same effect on beef and turkey meat. The depletion of the carnosine content in meat during domestic cooking can be primarily attributed to the loss in cooking juices due to the high water solubility of carnosine (Purchas et al., 2004; Peiretti et al., 2012). In contrast, no significant depletion of carnosine during cooking was observed by Park et al. (2005). Additionally, the retention value for carnosine in KNC meat was 81% in the present study, with a higher retention in leg meat (85%) than breast meat (78%; data not shown). Retention values for the same bioactive compounds after boiling were reported by Purchas et al. (2004) for lamb meat (76%) and by Peiretti et al. (2012) for beef (50%) and turkey meat (60%). According to the pooled data in the present study, the carnosine content in meat from KNC depended on the meat type (breast and leg meat) and the state of the meat (raw and cooked meat), in order of significance.

Anserine Content

The anserine contents of breast and leg meat from KNC, both in raw and cooked meat, are given in Table 2. The KNC meat contained more anserine than carnosine, irrespective of the age of the bird, the meat type, or the state of the meat. This result agrees with previous findings that anserine was the principal histidyl dipeptide in poultry meat (Abe and Okuma, 1995), and its content in chicken and rabbit meat was more than double the amount of carnosine (Peiretti et al., 2011). Chan and Decker (1994) stated that the anserine content in muscle is influenced by muscle type and animal age. However, differences in anserine content were not observed among the age groups of KNC tested in the current study ($P > 0.05$), except in raw leg meat. The anserine content of raw leg meat decreased ($P < 0.05$) as the age of the KNC increased. The 10-wk-old KNC had the highest anserine content in raw leg meat, whereas the 14-wk-old KNC had the lowest amounts (Table 2). However, no significant difference in the anserine content of raw leg meat was found in 11- and 13-wk-old KNC. Lee et al. (2011) showed that the BW of KNC increased with the age of the birds. Recently Jung et al. (2013) found no correlation between the BW and anserine content in meat from 5 lines of KNC.

Table 2. Anserine content (mg/100 g) of the raw and cooked meat of Korean native chicken at different ages

	Raw meat			Cooked meat			Analyzed value		
Item (mg/100 g)	Breast	Leg	SEM[1]	Breast	Leg	SEM[1]	Meat type	State of meat	Age
Age (wk)									
10	635.60[a]	314.40[b,x]	32.43	540.83[a]	190.65[b]	48.50			
11	553.20[a]	243.60[b,xy]	28.72	376.38[a]	208.94[b]	48.96			
12	667.20[a]	227.60[b,xy]	48.86	500.77[a]	193.90[b]	89.34			
13	570.40[a]	231.60[b,xy]	36.71	521.11[a]	207.60[b]	56.59			
14	618.80[a]	207.60[b,y]	22.91	426.22[a]	182.09[b]	78.33			
SEM[2]	38.70	30.94		102.32	14.73				
P-value							<0.0001	0.0007	0.3703
F-value							156.08	12.92	1.09

[a,b]Mean values in the same row with different superscripts within the same state of meat differ significantly ($P < 0.05$).

[x,y]Mean values in the same column with different superscripts differ significantly ($P < 0.05$).

[1]n = 20.

[2]n = 50.

Significantly higher contents of anserine were found in the breast meat of KNC compared with leg meat, regardless of the age of the KNC and the state of the meat. The breast meat contained an average of 541.05 mg of anserine per 100 g, whereas the leg meat had 220.80 mg/100 g. Similar results were reported by other researchers regarding higher contents of anserine in white muscles than dark muscles of chicken (Plowman and Close, 1988) and turkey (Davies et al., 1978). Jung et al. (2013) reported that anserine has the potential to act as a buffer against proton produced by anaerobic glycolysis in breast muscle, similar to carnosine (Dunnett and Harris, 1995).

As far as the anserine content of KNC meat is concerned, a depletion was detected during cooking ($P < 0.05$; Table 2). Raw meat had greater anserine contents (average of 427.00 mg/100 g) than cooked meat (average of 334.85 mg/100 g; $P < 0.05$). This result agrees with the findings of Peiretti et al. (2012), who found a greater loss in the anserine content of beef and turkey meat during boiling in water. The high water solubility of anserine, similar to carnosine, caused greater losses of this bioactive compound with the cooking juices during cooking (Purchas et al., 2004; Peiretti et al., 2012). Regarding the retention value of anserine in KNC meat during cooking, a higher value (82%) was reported in the present study (data not shown) compared with that reported by Peiretti et al. (2012). Similar to carnosine, the leg meat showed better retention (86%) than breast meat (78%; data not shown). Peiretti et al. (2012) reported much lower retention values for anserine in beef (30%) and turkey meat (65%) during boiling compared with the current data. However, the same authors revealed that microwave cooking was a good cooking method to preserve carnosine and anserine in meat during cooking, as it caused a smaller loss of both bioactive peptides due to the formation of hard surface layer on the meat. According to the pooled data in the present study, the anserine content in KNC meat is influenced by the meat type (breast and leg meat) and the state of meat (raw and cooked meat), in order of significance.

Creatine Content

Table 3 shows the creatine contents of breast and leg meat of KNC as affected by the age of the bird and domestic cooking. Accordingly, the age of the KNC had no significant effect on the creatine content of both types of meat and cooked state. Jung et al. (2013) showed that no correlation exists between the creatine content of breast meat and the BW of KNC. With increasing age, the BW of KNC increased in the current study ($P < 0.05$; data not shown). The state of the meat had an effect on the creatine content of the KNC meat ($P < 0.05$). In this regard, the creatine content of raw meat (average of 377.12 mg/100 g) was significantly higher than that of cooked meat (average of 233.06 mg/100 g). This loss in creatine content is mainly due to a nonenzymatic conversion of creatine to creatinine during cooking (Purchas et al., 2004; Mora et al., 2010). Similar reductions in muscle creatine content during cooking were found by Purchas et al. (2004) and Mora et al. (2010). Compared with histidyl dipeptides, the retention of creatine in KNC meat after cooking was 64%. Purchas et al. (2004) reported a similar retention value in boiled lamb meat (57%).

A large amount of phosphocreatine is required for immediate regeneration of adenosine triphosphate in type II muscle fibers, which resulted in higher levels of creatine in glycolytic muscles (Wyss and Kaddurah-Daouk, 2000; Mora et al., 2010). However, the creatine contents of breast and leg meat from KNC were comparable during the current study, both in the raw and cooked states, as well as at each age ($P > 0.05$); although the compositions of the muscle fiber types between the 2 muscles have clear differences. In agreement with our results, Jung et al. (2013) showed no significant differences in the creatine content between breast and thigh meat from KNC. In another study, it was reported that the creatine contents of type I and II muscle fibers did not differ, even though higher phosphocreatine contents existed in the type II muscle fibers of rodents compared with type I muscle fibers (Kushmerick et al., 1992)

Table 3. Creatine content (mg/100 g) of the raw and cooked meat of Korean native chicken at different ages

	Raw meat			Cooked meat			Analyzed value		
Item (mg/100 g)	Breast	Leg	SEM[1]	Breast	Leg	SEM[1]	Meat type	State of meat	Age
Age (wk)									
10	382.00	396.00	22.73	286.19	231.32	18.77			
11	380.00	369.60	22.38	238.86	236.39	36.19			
12	398.40	386.00	16.11	269.98	241.37	24.74			
13	374.40	350.80	24.23	266.69	204.61	20.23			
14	362.00	372.00	17.28	203.13	202.06	17.40			
SEM[2]	15.06	25.27		31.17	14.92				
P-value							0.0962	<0.0001	0.0813
F-value							2.86	187.78	2.19

[1]n = 20.
[2]n = 50.

Betaine Content

According to Table 4, all 3 factors tested in the current study affected the betaine content of KNC meat ($P < 0.05$). With the increasing age of KNC, the betaine content decreased in raw breast and cooked leg meat ($P < 0.05$). Meat from 10- and 11-wk-old KNC showed significantly higher betaine contents in their raw breast meat than did 13-wk-old birds. With regard to the betaine content of cooked leg meat, 12- and 14-wk-old KNC had lower values than 10- and 11-wk-old birds ($P < 0.05$). Significantly greater contents of betaine were found in the leg meat of KNC than the breast meat, irrespective of the age of the bird and the state of the meat (Table 4). The average betaine contents of the breast and leg meat were 4.26 and 10.75 mg/100 g, respectively (data not shown). It has been reported in the USDA database for the choline content of common foods that broiler drumstick and thigh meat contained higher betaine content than breast meat (Patterson et al., 2008).

The betaine content of KNC meat was higher in its raw state than in the cooked state (Table 4; $P < 0.05$). The average betaine contents of raw and cooked meat were 8.81 and 6.20 mg/100 g, respectively (data not shown). Similar results were reported by Patterson et al. (2008), showing higher levels of betaine in raw broiler meat and the raw liver and heart of turkey than the respective cooked states. During a study to detect the betaine contents of common food, de Zwart et al. (2003) revealed that the level of betaine varied depending on the processing method or the cooking method. Moreover, cooking affected the betaine content of food, with larger depletions (60 to 80%) during boiling and only small to medium losses during steaming. However, no significant losses were found during baking, microwaving, or frying. In another study, roasted chicken meat had 5.09 mg of betaine per 100 g of meat, which is closer to the results of the present study (Zeisel et al., 2003). The loss of betaine content during cooking was expected during the present study because betaine is also a highly water-soluble molecule (de Zwart et al., 2003). Boiling inhibited phospholipase D activity, which is responsible for the conversion of phosphatidylcholine to phosphatidic acid and choline (Zeisel et al., 2003). Hence, low choline production during boiling can lead to a reduction in the betaine content. The retention value of betaine in KNC meat during boiling was 79% (data not shown). Breast meat had an almost complete recovery of betaine (95%) compared with leg

Table 4. Betaine content (mg/100 g) of the raw and cooked meat of Korean native chicken at different ages

	Raw meat			Cooked meat			Analyzed value		
Item (mg/100 g)	Breast	Leg	SEM[1]	Breast	Leg	SEM[1]	Meat type	State of meat	Age
Age (wk)									
10	$5.05^{b,x}$	13.50^{a}	1.16	4.73^{b}	$10.33^{a,x}$	0.34			
11	$5.00^{b,xy}$	13.24^{a}	0.51	4.60^{b}	$8.30^{a,y}$	0.52			
12	$4.42^{b,xyz}$	14.06^{a}	0.73	4.22^{b}	$7.36^{a,z}$	0.37			
13	$3.55^{b,z}$	13.48^{a}	0.81	3.47^{b}	$7.93^{a,yz}$	0.17			
14	$3.84^{b,yz}$	11.95^{a}	0.20	3.71^{b}	$7.36^{a,z}$	0.22			
SEM[2]	0.36	0.99		0.41	0.26				
P-value							<0.0001	<0.0001	0.0027
F-value							616.43	99.58	4.87

[a,b]Mean values in the same row with different superscripts within the same state of meat differ significantly ($P < 0.05$).
[x–z]Mean values in the same column with different superscripts differ significantly ($P < 0.05$).
[1]n = 20.
[2]n = 50.

meat (63%). According to the pooled data of the current study, it can be stated that the betaine content in KNC meat is influenced by the meat type (breast and leg meat), the state of the meat (raw and cooked meat), and the age of the bird, in order of significance.

Carnitine Content

In the current study, the meat type and the state of the meat showed significant effects on the carnitine content of KNC meat (Table 5). However, the carnitine content did not differ due to the age of the bird ($P > 0.05$). The carnitine content of the leg meat was higher than that of the breast meat in the raw state at each age but not in the cooked state ($P < 0.05$). Previous studies regarding KNC have shown that the leg meat of KNC contained greater fat content than did the breast meat (Jeon et al., 2010; Jayasena et al., 2013). Rigault et al. (2008) calculated the correlation between the carnitine content and the fat content of beef cuts and showed that high-fat meat cuts are higher in carnitine than lean cuts. Constantin-Teodosiu et al. (1996) showed that carnitine buffered excess acetyl group formation during exercise. Although this happened in both type I and II fibers, type I fibers contained greater mitochondrial contents and produced higher levels of acetyl groups. Therefore, muscles rich in type I fibers, such as leg muscles, need higher carnitine contents to buffer the excess acetyl groups. This was further confirmed by the greater accumulation of acetylcarnitine in type I fibers during prolonged exercise ($P < 0.05$).

Raw meat contained significantly greater amounts of carnitine compared with cooked meat, at average levels of 9.92 and 7.10 mg/100 g of meat, respectively ($P < 0.05$). By contrast, Rigault et al. (2008) demonstrated that cooking by means of frying, boiling, grilling, baking, microwave cooking, or steaming did not change the carnitine content of 7 beef cuts or salmon ($P > 0.05$). However, the cooking time used by these authors was short, ranging from 2 to 10 min, and this might be the reason for the higher retention of carnitine in the beef cuts and salmon. In contrast, KNC meat was boiled for 40 min in the current study, representing the long-term boiling used in local soups, such as *samgyetang* and *baeksuk*. Hence, the depletion of carnitine in the present study can be attributed to the longer boiling period, in addition to the higher water solubility of this bioactive compound (Arslan et al., 2003). During the same study, Rigault et al. (2008) showed that smoking the salmon, which was usually performed for several days at various temperatures, modified the carnitine content ($P < 0.05$), with a very low retention value of 32% compared with boiling (92%). The retention value of carnitine in KNC meat during boiling was similar to that of betaine (77%; data not shown). The leg meat showed greater depletion, with a lower retention value (57%), whereas breast meat had an almost complete recovery (97%). According to the pooled data, the state of the meat (raw and cooked meat) and the meat type (breast and leg meat) affected the carnitine content in KNC meat.

In summary, KNC had a considerable amount of these bioactive compounds in the meat. The effect of age on these compounds was only observed for betaine ($P < 0.01$). Compared with leg meat, the breast meat of KNC had significantly higher contents of carnosine and anserine, but lower amounts of betaine and carnitine, most of which can be attributed to the differences in the composition of muscle fibers between the 2 muscles. Depletion in the content of all bioactive compounds occurred during the domestic cooking process, which was primarily due to the higher water solubility of these compounds. It can be concluded that the findings of the current study are important because they provide useful information regarding the availability and amounts of bioactive compounds in one of the most popular indigenous chicken breeds in the world and regarding a few determinants that affect the content of these compounds. Further studies are being conducted to compare the contents of these bioactive compounds in KNC with those of commercial broilers.

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Table 5. Carnitine content (mg/100 g) of the raw and cooked meat of Korean native chicken at different ages

	Raw meat			Cooked meat			Analyzed value		
Item (mg/100 g)	Breast	Leg	SEM[1]	Breast	Leg	SEM[1]	Meat type	State of meat	Age
Age (wk)									
10	7.96[b]	11.59[a]	0.94	7.37	6.51	0.69			
11	7.71[b]	11.92[a]	0.76	7.22	6.77	0.70			
12	7.78[b]	12.29[a]	0.67	6.32	7.08	0.50			
13	7.49[b]	13.37[a]	0.46	8.08	7.24	0.52			
14	7.16[b]	11.99[a]	0.51	7.47	6.94	0.51			
SEM[2]	0.85	0.63		0.79	0.29				
P-value							<0.0001	<0.0001	0.5501
F-value							49.00	87.54	0.77

[a,b]Mean values in the same row with different superscripts within the same state of meat differ significantly ($P < 0.05$).

[1]n = 20.

[2]n = 50.

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